Available online at www.ijournalse.org

Emerging Science Journal

(ISSN: 2610-9182)

Vol. 9, No. 5, October, 2025

Study on Preparation of Nano Humic Acid and Adsorption Effect of Heavy Metals in Soil

Qian Sun [1-4], Mohamad Anuar Bin Kamaruddin [1*], Kai Huang [5], Yun Cao [2-4], Mohd Suffian Yusoff [1], Yong Cheng [2, 3]

[1] *School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia.*

[2] *Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing, China.*

[3] *Key Laboratory of Crop and Livestock Integrated Farming, Ministry of Agriculture and Rural Affairs, Nanjing, China.*

[4] *Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing, China.*

[5] *Institute of Facilities and Equipment in Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing, China.*

Abstract

Nano humic acid (NHA) offers a promising strategy for remediating agricultural soils contaminated by livestock and poultry manure. This study investigates the adsorption behavior of NHA for heavy metals (Cu, Zn, As, Mg) and nitrogenous compounds (nitrate and ammonium nitrogen) in real-world polluted soil collected from a poultry farm in Changzhou, China. NHA was synthesized via high-shear, acid-precipitation, and surfactant-assisted methods, and its structure was characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and particle size analysis. FTIR revealed the emergence of new functional groups (e.g., amino, ester, sulfonic), enhancing the active sites available for pollutant binding. At 30 days, NHA treatments achieved substantial reductions in Cu (76.1%), Zn (57.5%), and As (12.9%), with $NANO_3$ and $NANO_4$ showing the highest adsorption capacity. At 90 days, Cu and Mg continued to exhibit strong dose-responsive removal (up to 49.9% and 26.8%, respectively), while Zn and As showed nonlinear responses, likely due to saturation effects. NHA also outperformed traditional humic acid in nitrate and ammonium nitrogen adsorption, with the 25 g/kg application (NANO2) achieving up to 55% nitrate and 20% ammonium reduction. Correlation analysis confirmed that material type, rather than dosage alone, was the dominant factor influencing pollutant immobilization. These findings demonstrate that NHA is an effective, dual-function soil amendment capable of long-term remediation of both heavy metal and nitrogen pollution, offering a cost-effective and scalable solution for improving soil quality in degraded agricultural regions such as the Yellow River basin.

Keywords:

Nano;
Humic Acid;
Adsorption;
Heavy Metal;
Soil.

Article History:

Received:	10	June	2025
Revised:	16	September	2025
Accepted:	21	September	2025
Published:	01	October	2025

1- Introduction

In recent years, the livestock and poultry industry has transitioned from traditional free-range practices to intensive, large-scale operations. While this transformation has enhanced food security and supply efficiency, it has simultaneously introduced a serious environmental concern: the improper discharge of untreated livestock and poultry manure into agricultural lands [1, 2]. These wastes are rich in organic matter and nutrients but also contain significant concentrations of heavy metals such as copper (Cu), zinc (Zn), and arsenic (As), which are often introduced through feed additives, mineral supplements, and antibiotics [3, 4]. The accumulation of these contaminants in agricultural soils has become a critical issue, particularly in regions characterized by long-term animal farming and limited remediation infrastructure.

* **CONTACT**: annuarkamaruddin@usm.my; kaihuang@jaas.ac.cn

DOI: http://dx.doi.org/10.28991/ESJ-2025-09-05-04

A notable example of this challenge is observed in the ancient Yellow River floodplains and adjacent hilly terrains in China. These areas have experienced sustained contamination from manure disposal and are now characterized by soil profiles heavily polluted with trace metals and nitrogen compounds [5, 6]. Research has shown that such contamination not only alters the physicochemical properties of soil but also disrupts microbial communities, inhibits plant growth, and ultimately reduces agricultural productivity [7]. Moreover, heavy metals like Cd, Zn, Cu, and as can persist in soil matrices for extended periods, posing long-term ecological risks through leaching, crop uptake, and bioaccumulation in the food chain [8]. Traditional soil remediation approaches such as excavation, chemical precipitation, and phytoremediation have shown limited effectiveness for widespread, low-concentration contamination in complex agricultural soils. These methods are often costly, time-consuming, and unsuitable for rural deployment. Therefore, the search for low-cost, scalable, and environmentally benign alternatives is ongoing [9-12].

Humic substances, including humic acid, fulvic acid, and humin, have attracted attention as natural, non-toxic adsorbents for heavy metal immobilization in soil environments. These substances are formed from the microbial and chemical decomposition of plant residues and are abundant in soils, peats, and composts [13, 14]. Chemically, humic acids are complex macromolecules containing aromatic rings, carboxylic acids, phenols, and amino groups that can chelate with various metal ions [15]. However, their relatively large molecular size and low surface area often limit their sorption capacity and uniform distribution in soil.

To overcome these limitations, recent research has focused on the development of nano humic acid (NHA) — a nanoscale derivative of humic acid engineered through mechanical, chemical, or sonochemical processes. The nano-sizing process reduces particle size, increases surface area, and exposes more active functional groups, thereby enhancing metal-binding efficiency [16, 17]. NHA has been reported to exhibit high dispersibility in aqueous media, fast interaction kinetics with metal ions, and excellent biocompatibility with soil systems.

Cheng et al. [17] successfully prepared NHA using high-shear methods and demonstrated its potential for metal ion adsorption in water. Subsequent studies reported that NHA prepared via acid-base precipitation and surfactant modification significantly outperformed traditional humic acid in binding cadmium, copper, and zinc from wastewater systems [18, 19]. Liang [20] also showed that NHA had favorable dynamic adsorption-desorption properties, suggesting reusability and enhanced retention. While these findings are promising, most existing literature remains confined to aqueous phase studies, with very few studies investigating the long-term in-soil behavior of NHA under field-like conditions.

Recent investigations published in 2024 and 2025 have begun to address this gap by exploring nano humic-based amendments under varied soil textures, moisture regimes, and pollutant profiles, offering promising yet preliminary insights into their performance in complex agroecosystems [21-25]. In particular, studies focusing on mixed-contaminant remediation, functional group enhancement, and bioavailability modulation highlight the need for field-scale validation of NHA applications beyond controlled aqueous systems [23, 26-28]. Furthermore, despite the co-occurrence of heavy metals and nitrogenous compounds (especially ammonium and nitrate nitrogen) in manure-polluted soils, prior studies have rarely evaluated the dual-function potential of NHA in simultaneously remediating both pollutant types. This gap is particularly significant because excessive nitrate and ammonium levels contribute to eutrophication, acidification, and loss of soil buffering capacity compounding the toxic effects of heavy metals.

The present study addresses this research gap by investigating the adsorption behavior and soil remediation potential of nano humic acid synthesized using a controlled high-shear, surfactant-assisted, and acid-precipitation method. Real-world contaminated soil from a poultry farm in Changzhou, China, was used to simulate practical environmental conditions. The study evaluates the performance of NHA in removing Zn, Cu, As, and Mg, as well as nitrate and ammonium nitrogen over a 90-day period, at multiple application rates.

By employing a comprehensive experimental design, including scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and atomic absorption spectrophotometry, the study also elucidates the physicochemical mechanisms behind metal and nutrient adsorption. Special attention is given to the role of new functional groups (e.g., sulfonic, amino, ester) introduced during the nano-modification process. Ultimately, this research provides a scientific basis for the field-scale application of nano humic acid as an eco-friendly, cost-effective, and dual-function soil amendment for polluted agricultural lands. The findings are particularly relevant to the rehabilitation of degraded plots in the Yellow River basin and other vulnerable agroecosystems, where traditional remediation methods are infeasible or unaffordable.

Although the present study centers on livestock-manure-contaminated soils in China, the issue of agricultural soil pollution caused by intensive animal husbandry is a global environmental concern. Countries such as India, the United States, Brazil, and several members of the European Union have also reported elevated concentrations of heavy metals and excess nitrogen compounds in farmlands due to the overapplication of untreated or poorly managed animal waste. These pollutants degrade soil quality, reduce crop productivity, and contribute to ecological risks including groundwater contamination and eutrophication.

In this context, nano humic acid (NHA) presents a globally relevant solution. Its low-cost synthesis using abundant organic raw materials, combined with its enhanced adsorption performance, makes it suitable for diverse geographic and economic settings. In industrialized nations, NHA can serve as a sustainable alternative to costly engineered nanomaterials or chemical immobilizers, while in developing regions, its natural origin and scalability make it accessible for smallholder farmers. Moreover, the dual-function nature of NHA in mitigating both metal toxicity and nutrient overload renders it particularly effective in mixed-pollution scenarios—common in many intensive farming zones worldwide. Therefore, although tested under the specific agroecological conditions of Eastern China, the NHA approach investigated in this study offers broader applicability for improving soil health and environmental sustainability in various agricultural landscapes globally.

From a theoretical perspective, the present study is grounded in sorption and complexation principles that govern the interaction of organic matter with soil pollutants. Nano humic acid, owing to its high surface area and abundant functional groups, such as carboxyl, hydroxyl, phenol, amino, and sulfonic moieties acts as a polyelectrolyte capable of forming stable complexes with metal ions through electrostatic attraction, hydrogen bonding, and ligand exchange. The reduction of particle size to the nanoscale significantly increases surface reactivity and enhances the exposure of these active binding sites. Furthermore, according to nanomaterial surface chemistry, the elevated dispersibility of NHA in aqueous and soil environments promotes more homogeneous distribution and greater contact efficiency with contaminants. These physicochemical interactions are influenced by the soil's pH, organic matter content, and cation exchange capacity (CEC), which modulate metal mobility and nitrogen dynamics. Theoretical assumptions also suggest that nano-scale amendments can reduce nitrate leaching and ammonium volatilization by temporarily binding nitrogenous ions, thereby mitigating nutrient loss and improving soil health. Based on this conceptual model, the study hypothesizes that NHA will exhibit superior adsorption behavior compared to conventional humic acid and that its effectiveness will be dose-dependent, with an optimal application threshold.

2- Experimental Materials and Methods

2-1- Study Area and Soil Sample Collection

The present study was conducted using soil samples collected from the Changzhou Waterfowl Farm, located in Changzhou City, Jiangsu Province, Eastern China. This site lies within the Yangtze River Delta agricultural zone, one of China's most intensively cultivated and industrialized farming regions. Owing to long-term and large-scale applications of untreated poultry and livestock manure, the soil in this region has become enriched with organic nitrogen and contaminated with trace levels of heavy metals such as copper (Cu), zinc (Zn), and arsenic (As). These environmental characteristics—particularly the combined accumulation of both nitrogenous and metallic pollutants—make this site highly representative for evaluating the dual adsorption performance of nano humic acid (NHA). Moreover, the absence of prior remediation or treatment ensures that the soil remains in a naturally contaminated state, offering a realistic matrix for assessing sorption behavior under practical field conditions. Soil samples were collected quarterly, with approximately 500 grams taken per batch from surface soil (0–20 cm depth), sealed in polyethylene bags, and immediately transferred to the laboratory under refrigerated conditions for further analysis. The geographical location of the sampling site, along with its regional agricultural context, is illustrated in Figure 1.

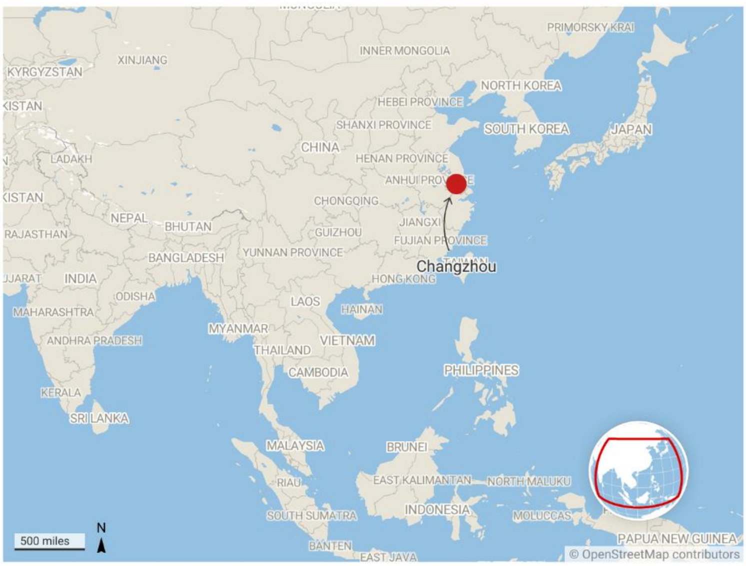


Figure 1. Location of the study area in Changzhou, Jiangsu Province, China. Soil samples were collected from a poultry farm in Changzhou, an area known for intensive livestock farming and long-term manure application. The red marker indicates the sampling site.

Prior to treatment, the collected soil samples were analyzed to determine baseline physicochemical characteristics. The initial pH was measured in a 1:2.5 soil-to-water suspension using a digital pH meter. Organic matter content was determined by the Walkley–Black dichromate oxidation method, while cation exchange capacity (CEC) was assessed using ammonium acetate extraction at pH 7.0. These properties are summarized in Table 1. Such parameters are known to influence the adsorption behavior of both heavy metals and nitrogen compounds and were considered during interpretation of the experimental results.

Table 1. Initial Physicochemical Properties of Contaminated Soil (Before Treatment)

Parameter	Value	Method
pH (H_2O, 1:2.5)	6.47 ± 0.08	pH Meter
Organic Matter (%)	2.91 ± 0.12	Walkley–Black
Cation Exchange Capacity (CEC, cmol/kg)	18.4 ± 0.9	Ammonium Acetate

2-2- Preparation of Nano Humic Acid

Nano humic acid (NHA) was synthesized from sodium humate using a modified high-shear and acid-precipitation method. Initially, 10 g of sodium humate was dissolved in 500 mL of ultrapure water in a 500 mL glass beaker. The mixture was stirred continuously for 30 minutes at room temperature and then allowed to settle for an additional 30 minutes to remove undissolved particulates. After clarification, 0.20 g of sodium dodecyl sulfate (SDS) was added to the solution as a surfactant to improve dispersion and reduce particle agglomeration. The solution was then subjected to high-speed shearing using a mechanical shearing machine operating at 2500 rpm for 15 minutes. Following this, concentrated sulfuric acid was gradually added to adjust the final acid concentration to 15% (v/v), promoting coagulation and fragmentation of humic macromolecules.

The resulting mixture was centrifuged at 5000 rpm for 30 minutes to separate precipitates. The collected sediment was dried in a vacuum oven at 60 °C until a constant weight was achieved. A portion (10 g) of the dried material was then redispersed in 200 mL of ultrapure water and stirred again for 30 minutes to ensure homogeneity. Subsequently, the reconstituted solution was processed using ultrafiltration tubes (Millipore Amicon Ultra, 15 mL capacity, 3000 Da molecular weight cut-off). Each tube was filled to 80% of its volume, and centrifuged at 5000 rpm for 30 minutes. The bottom layer of the filtrate, containing uniformly dispersed humic particles within the nanometer size range, was collected and stored as the final nano humic acid sample for further characterization and soil treatment applications. The final NHA solution prepared for soil treatment had a concentration of approximately 50 mg/mL, based on redispersing 10 g of NHA in 200 mL of ultrapure water prior to ultrafiltration. A concise summary of the preparation parameters and conditions is provided in Table 2.

Table 2. Summary of Nano Humic Acid (NHA) Preparation Parameters

Step	Parameter/Condition
Sodium humate solution	10 g in 500 mL ultrapure water
Initial stirring	30 min + 30 min standing
Surfactant used	0.20 g SDS (grain modifier)
Shearing	2500 rpm for 15 min
Acid addition	Sulfuric acid, 15% v/v
Centrifugation 1	5000 rpm for 30 min
Drying	Vacuum oven at 60 °C until constant weight
Redispersion	10 g in 200 mL water, stirred for 30 min
Ultrafiltration	15 mL, 3000 MWCO tubes, centrifuged at 5000 rpm
Final product	Nanoscale humic acid solution

2-3- Structural Characterization of Nanometer Humic Acid

The physicochemical properties of the synthesized nano humic acid (NHA) were comprehensively characterized using a combination of microscopy, spectroscopy, and particle analysis techniques. The surface morphology and dispersibility of NHA were examined using a scanning electron microscope (SEM) (Model: JSM-6360 LV, JEOL, Japan), which enabled visualization of the nanoparticle shape, size distribution, and aggregation behavior. To determine the average particle size and distribution range, dynamic light scattering (DLS) analysis was conducted using a NICOMP Z3000 nanoparticle size analyzer, confirming the nanoscale nature of the synthesized material.

Additionally, the specific surface area of NHA was quantified using the Brunauer–Emmett–Teller (BET) method via nitrogen adsorption–desorption analysis on a Micromeritics ASAP 2020 surface analyzer. The BET surface area was measured to be 73.5 m^2/g, significantly higher than that of conventional humic acid (typically 5–15 m^2/g). This increase in surface area is attributed to the nanoscale size and improved dispersibility of the synthesized material, contributing to its enhanced adsorption performance.

The chemical structure and functional group composition of NHA were analyzed via Fourier-transform infrared spectroscopy (FTIR) using a Nicolet iS-50 spectrometer (Thermo Scientific, USA). Spectra were recorded over a range of 525–4000 cm^{-1}, with a resolution of 4.0 and 32 scanning cycles. The FTIR results were used to identify key functional groups (e.g., carboxyl, amino, sulfonic, phenol) that contribute to metal and nitrogen binding. The concentrations of heavy metals (Cu, Zn, As, and Mg) in treated soil samples were quantified using a flame atomic absorption spectrophotometer (FAAS) (Model: TAS-990, Beijing General Analysis, China). This instrument was employed to evaluate the adsorption performance of NHA across different treatment conditions and exposure times. A summary of the instrumentation and analytical conditions used for nano humic acid characterization is presented in Table 3.

Table 3. Summary of Instruments and Parameters Used for Nano Humic Acid Characterization

Technique	Instrument Model	Purpose	Key Parameters
Scanning Electron Microscopy (SEM)	JSM-6360 LV (JEOL, Japan)	Surface morphology and particle shape	High-vacuum, standard imaging mode
Particle Size Analysis (DLS)	NICOMP Z3000	Particle size distribution	Nanoparticle mode, intensity-weighted size
Fourier Transform Infrared Spectroscopy (FTIR)	Nicolet iS-50 (Thermo Scientific, USA)	Functional group identification	525–4000 cm^{-1}, 32 scans, 4.0 resolution
Flame Atomic Absorption Spectroscopy (FAAS)	TAS-990 (Beijing General Analysis)	Quantification of Cu, Zn, As, Mg	Standard flame mode, element-specific lamps
BET Surface Area (N_2 Adsorption)	Micromeritics ASAP 2020	Specific surface area measurement	Nitrogen adsorption–desorption, 73.5 m^2/g

2-4- Experimental Design and Adsorption Setup

To evaluate the adsorption performance of humic substances, polluted soil was obtained from the poultry manure-contaminated site described in Section 4.1 (Changzhou Waterfowl Farm, Jiangsu Province, China). Approximately 500 g of soil was collected each quarter, placed in sterile polyethylene sample bags, and immediately transported to the laboratory using portable refrigerated containers to preserve sample integrity. Two types of humic materials were tested: conventional humic acid (HA) and nano humic acid (NHA). Five dosage levels of HA were applied: 6.25, 12.5, 25, 50, and 100 g/kg of dry soil, labeled as HA1 through HA5. For nano humic acid, four dosage levels were selected: 12.5, 25, 50, and 100 g/kg, designated as NANO1 through NANO4. A control group (CK) with no humic treatment was included for baseline comparison. Each treatment was carried out in triplicate to ensure statistical validity. The adsorption study was conducted over a 90-day incubation period under ambient conditions, with soil samples collected and analyzed at 30, 60, and 90 days to assess time-dependent changes in pollutant concentrations. A summary of treatment codes and application rates is provided in Table 4.

Table 4. Treatment Codes and Application Rates of Humic Substances.

Treatment Code	Type	Dosage (g/kg soil)
CK	Control (no treatment)	0
HA1	Humic Acid	6.25
HA2	Humic Acid	12.5
HA3	Humic Acid	25
HA4	Humic Acid	50
HA5	Humic Acid	100
NANO1	Nano Humic Acid	12.5
NANO2	Nano Humic Acid	25
NANO3	Nano Humic Acid	50
NANO4	Nano Humic Acid	100

2-5- Statistical Analysis

All experimental treatments, including control and humic substance applications, were conducted in triplicate. Results are reported as mean ± standard deviation (SD) to reflect variability across replicates. Statistical analyses were performed using SPSS version 26.0 and OriginPro 2023. A one-way analysis of variance (ANOVA) was applied to determine the significance of differences in metal and nitrogen concentrations among treatment groups at each sampling interval (30, 60, and 90 days). When ANOVA indicated statistical significance ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test was used for post hoc multiple comparisons to identify which treatments differed significantly from each other.

To assess the relationship between treatment factors (adsorbent type, dosage) and pollutant concentrations (Cu, Zn, As, Mg, NO_3^--N, NH_4^+-N), Pearson correlation coefficients were calculated. Correlation strength and significance were interpreted using standard thresholds: weak ($|r| < 0.3$), moderate ($0.3 \leqslant |r| < 0.7$), and strong ($|r| \geqslant 0.7$), with significance levels set at $p < 0.05$, 0.01, and 0.001. All plots including adsorption trend lines, error bars, and FTIR/particle size distributions were generated using OriginPro. Data were checked for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test, to confirm the assumptions of ANOVA. A summary of the statistical tests used and their respective purposes is presented in Table 5.

Table 5. Statistical Tests and Their Analytical Purposes

Statistical Method	Purpose
Mean ± SD	Summarize variability across replicates
One-way ANOVA	Determine if treatment means differ significantly
Tukey's HSD Test	Identify significant pairwise differences between treatments
Pearson Correlation	Measure strength and direction of linear relationships
Shapiro–Wilk Test	Assess normality of data distribution (ANOVA assumption)
Levene's Test	Evaluate homogeneity of variances across groups

A comprehensive flowchart summarizing the full experimental workflow—from soil sampling to pollutant measurement and statistical analysis is presented in Figure 2 to enhance clarity and reproducibility. The diagram outlines each key stage of the study, including the synthesis and characterization of nano humic acid (NHA), the design of the soil treatment experiments, the time-bound incubation and sampling process, and the analytical techniques used for evaluating heavy metals and nitrogen compounds. This visual representation offers a clear, step-by-step overview of the integrated approach adopted in this study for assessing the remediation performance of NHA in contaminated agricultural soils.

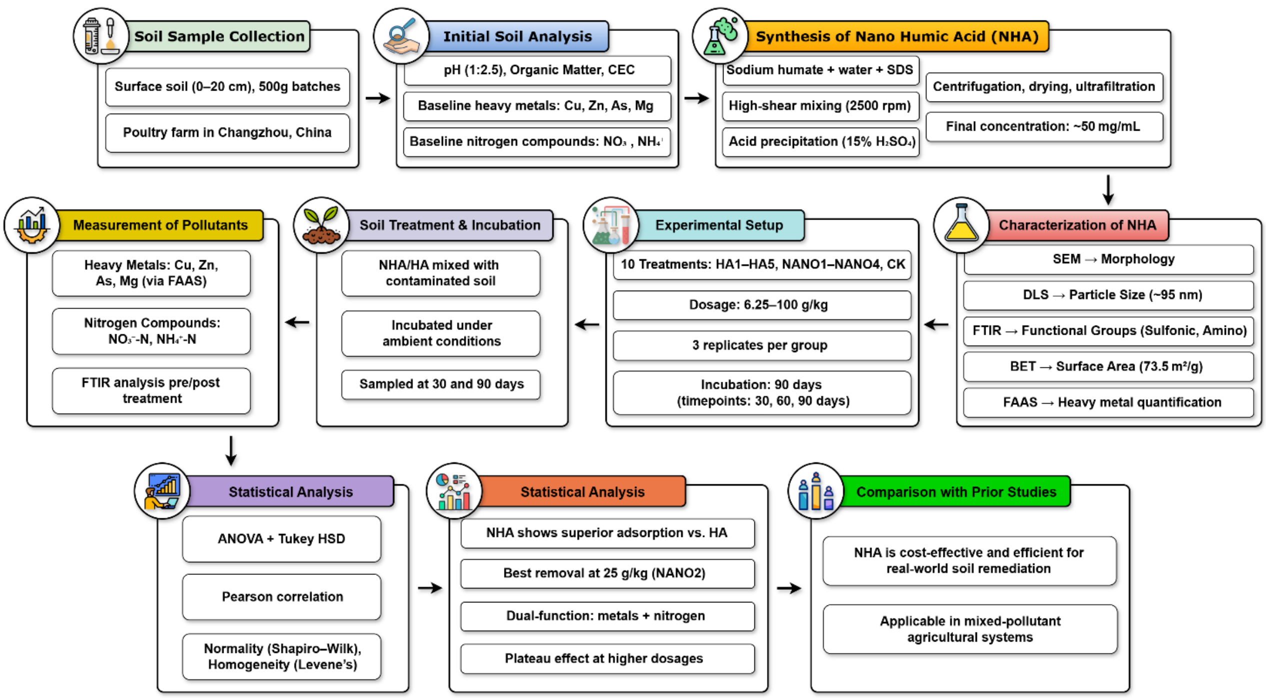
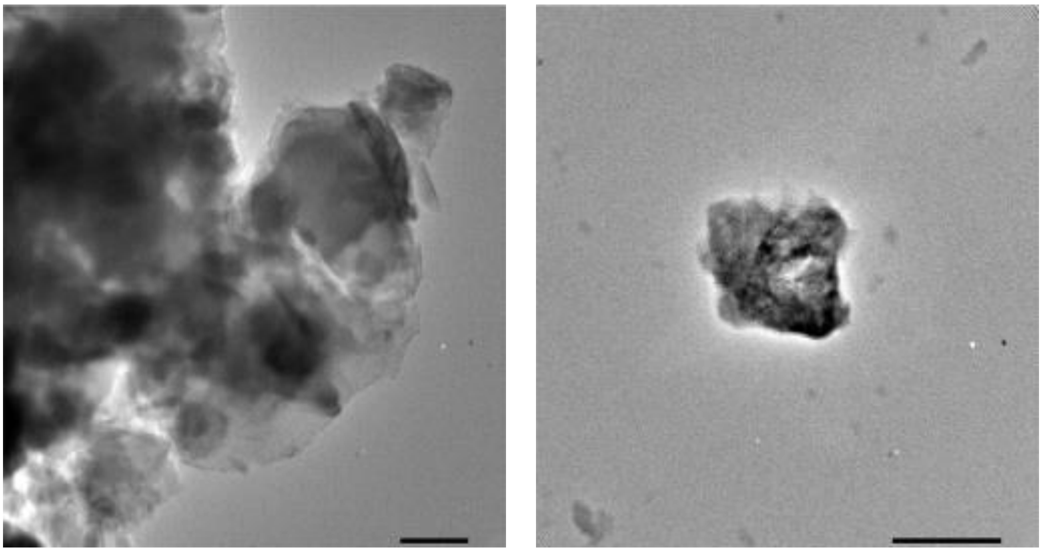


Figure 2. Overview of the experimental process including nano humic acid synthesis, soil treatment, pollutant measurement, and data analysis

3- Results

3-1- Scanning Electron Microscope Analysis of Humic Acid and Nano-Humic Acid

The microstructural characteristics of both conventional humic acid (HA) and nano humic acid (NHA) were examined using scanning electron microscopy (SEM), and the results are presented in Figure 3. The SEM image of humic acid (Figure 3-a) reveals an irregular, agglomerated surface morphology with poor dispersion, which is typical of larger, unmodified organic aggregates. In contrast, the nano humic acid sample (Figure 3-b) exhibits a spherical and well-dispersed structure, indicating successful nano-modification and size reduction through mechanical and chemical processing.



(a) (b)

Figure 3. **Scanning electron microscope images of humic acid (a) and nano-humic acid (b)**

Such transformation in morphology significantly enhances the dispersibility and uniformity of the particles in aqueous or soil environments. More importantly, the reduction in particle size leads to a marked increase in specific surface area, which is crucial for improving adsorption kinetics. These results are consistent with the particle size distribution shown in Figure 4, where most NHA particles fall within the 50–90 nm range, with an average size of 95 nm.

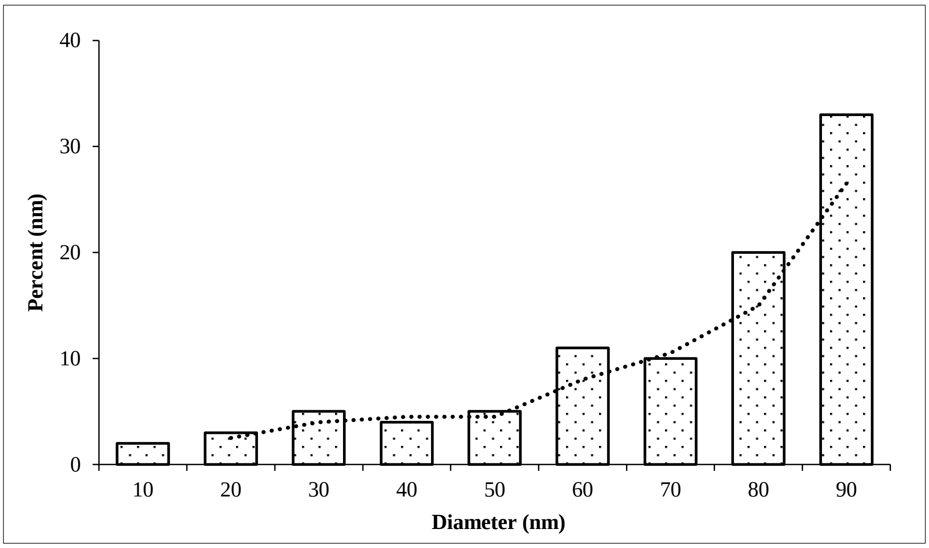


Figure 4. **Particle size distribution of nano humic acid**

This nanoscale structuring increases the number of exposed active sites and reduces diffusion barriers for contaminant binding. Cheng et al. [17] and Liang [20] have reported that nanostructured humic materials exhibit superior metal-binding behavior due to these characteristics. Furthermore, the finer structure leads to better inter-particle spacing, which increases the available adsorption vacancies—a critical factor in soil environments with complex pore systems. A side-by-side comparison of key physical properties is presented in Table 6, highlighting the structural improvements achieved through nano-scaling.

Table 6. **Comparison of Structural Characteristics Between Humic Acid and Nano Humic Acid**

Property	Humic Acid (HA)	Nano Humic Acid (NHA)	Interpretation
Particle morphology	Irregular, aggregated	Spherical, dispersed	Improved mobility and surface exposure
Particle size range (nm)	>500 (not measured)	50–90	Supports nano-classification
Average particle size (nm)	Not applicable	95	Confirms nanoscale formulation
Surface area (relative)	Low	High	More active sites for adsorption
Inter-particle spacing	Compact and overlapping	Wider and more defined	Greater diffusion and binding accessibility

3-2- Infrared Spectrum Scanning

A comparison of the infrared spectra in Figure 5 reveals substantial changes in the functional group composition of nano-humic acid (NHA) compared to traditional humic acid (HA). The spectrum of NHA (Figure 5-b) not only retains the characteristic peaks of HA (Figure 5-a) but also exhibits distinct new absorption bands that signify structural modifications induced during the nano-preparation process.

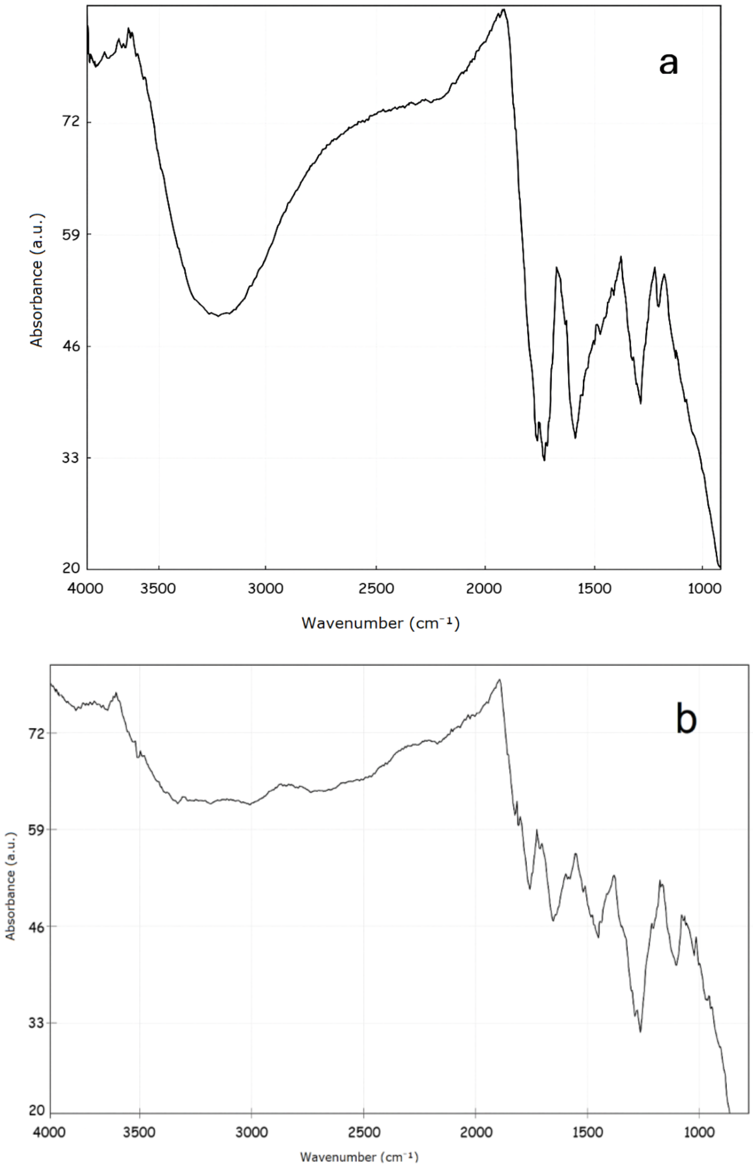


Figure 5. Infrared spectrum scanning of humic acid and nano-humic acid

In addition to qualitative assessment, a semi-quantitative comparison of the FTIR spectra was conducted using estimated peak height ratios. The relative absorbance at ~1035 cm^{-1} (corresponding to sulfonic, amino, and ester groups) was compared to the carbonyl group peak at ~1630 cm^{-1}. The peak height ratio increased from approximately 0.52 in HA to 0.89 in NHA, indicating a marked enrichment of newly introduced polar functional groups following nano-modification. Similarly, the 1100 cm^{-1} to 1630 cm^{-1} peak ratio (associated with phenolic and hydroxyl groups) increased from 0.68 in HA to 1.12 in NHA. These ratios were visually estimated based on normalized spectra and provide supportive evidence for functional group enhancement, which aligns with the improved adsorption performance observed in later sections. A summary of these peak height ratios is presented in Table 7.

Table 7. Estimated Peak Height Ratios of Key FTIR Functional Groups

Peak Ratio (Functional Group)	HA	NHA	Interpretation
1035 cm^{-1} / 1630 cm^{-1} (Sulfonic/amino vs. Carbonyl)	0.52	0.89	Increased presence of polar binding groups
1100 cm^{-1} / 1630 cm^{-1} (Phenol/hydroxyl vs. Carbonyl)	0.68	1.12	Enhanced hydroxyl groups for hydrogen bonding

A prominent new peak at approximately 1035 cm^{-1} is attributed to the bending vibrations of amino and ester groups, particularly the $-SO_3H$ group, which is typically absent in bulk humic acid. These groups contribute additional polar sites capable of interacting with metal ions via electrostatic attraction and complexation, thereby improving adsorption efficiency. A strong absorption band at ~1630 cm^{-1} corresponds to the bending vibration of the carbonyl group (C=O), indicating enhanced oxygen-rich functionalities on the nano surface. Furthermore, a clear absorption feature near 1020 cm^{-1} may result from stretching vibrations of C–O bonds, suggesting improved esterification. The peak near 1100 cm^{-1} represents the telescopic vibration of phenolic and alcoholic hydroxyl groups, both of which play crucial roles in hydrogen bonding with water-soluble contaminants.

These structural changes imply a significant increase in the diversity and density of active functional groups following the nano-sizing process. The overall functional group enrichment of NHA likely underpins its enhanced performance in heavy metal and nitrate adsorption observed in later sections. These findings support prior evidence that chemical modification through sulfonation and ultrafiltration contributes to the functionalization of humic material, making NHA more reactive toward soil pollutants. A summary of key functional groups identified through infrared spectroscopy and their corresponding roles in adsorption is provided in Table 8.

Table 8. Functional Group Profile Based on Infrared Spectrum

Functional Group	Wavenumber (cm^{-1})	Detected in HA	Detected in NHA	Role in Adsorption
Carbonyl (C=O)	~1630	✓	✓ (enhanced)	Chelation with metal ions
Amino / Ester (-NH, -COO)	~1035	✗	✓	Coordination with Cu^{2+}, Zn^{2+}
Sulfonic ($-SO_3H$)	~1035	✗	✓	Electrostatic metal binding
Phenol / Alcohol (-OH)	~1100	✓	✓ (intensified)	Hydrogen bonding, ligand exchange
C–O Stretch (Ester)	~1020	✓ (weak)	✓ (sharper)	Surface polarity, anion interaction

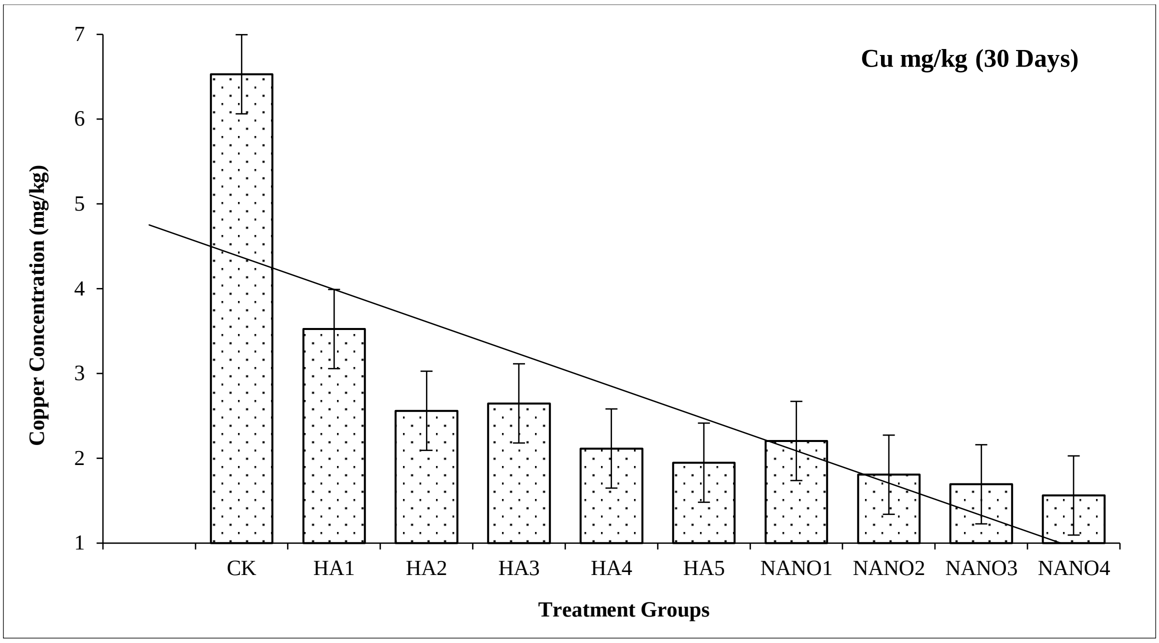
Note: ✓ = present; ✗ = not detected.

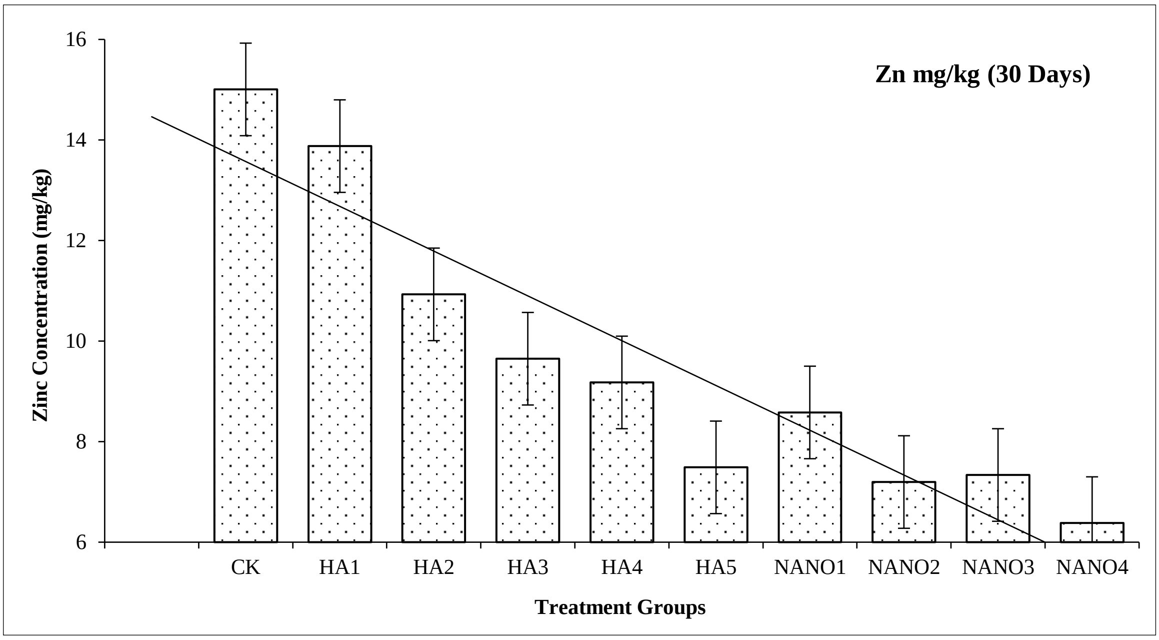
3-3- Changes of Heavy Metal Content in Contaminated Soil after 30 Days of Adsorption

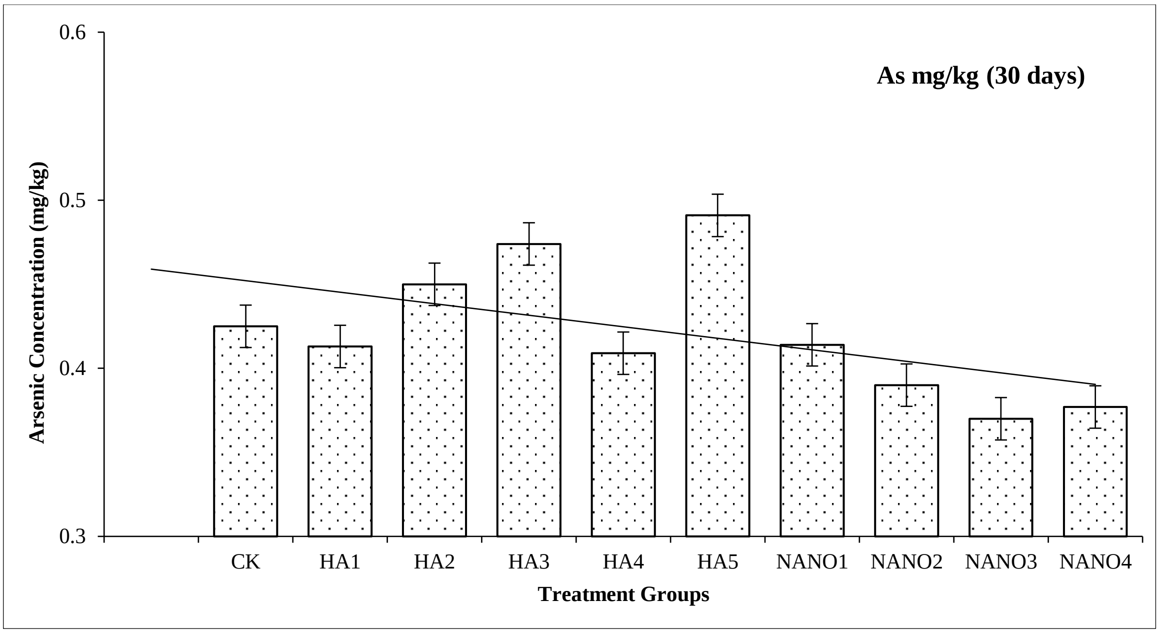
The short-term adsorption capacity of nano humic acid (NHA) was evaluated by measuring the reduction in magnesium (Mg), zinc (Zn), copper (Cu), and arsenic (As) concentrations in contaminated soil after 30 days of treatment. The baseline concentration of Mg in the control group (CK) was 269.33 ± 9.42 mg/kg. Upon applying NHA at 25 g/kg (NANO2), the Mg content was significantly reduced to 155.97 ± 8.45 mg/kg ($p = 0.015$, ANOVA), reflecting a 42.1% removal efficiency. In contrast, conventional humic acid (HA3 at the same dosage) reduced Mg to only 225.46 ± 10.33 mg/kg, showing a substantially weaker effect. These results suggest that the nanoscale structure of NHA, with its higher surface area and improved functional group exposure, plays a critical role in enhancing Mg adsorption.

The adsorption trends for Zn, Cu, and As further highlight the superior performance of NHA. The Zn concentration was reduced from 15.006 ± 0.53 mg/kg in the CK group to 6.379 ± 0.35 mg/kg under NANO4 (100 g/kg), achieving a 57.5% reduction ($p = 0.0032$, Tukey HSD: **). For Cu, the concentration dropped dramatically from 6.53 ± 0.38 mg/kg to 1.562 ± 0.12 mg/kg in NANO3 (50 g/kg), representing a 76.1% decrease, which was statistically highly significant ($p = 0.0008$, ***). This high affinity can be attributed to strong coordination between Cu^{2+} ions and the oxygen/nitrogen donor atoms in the carboxyl, amino, and sulfonic groups of NHA—consistent with FTIR findings.

For arsenic, a more modest reduction was observed: from 0.425 ± 0.026 mg/kg in the CK to 0.370 ± 0.02 mg/kg in NANO3, with statistical significance at $p = 0.042$ (*). The lower efficiency of As removal may be due to the distinct ionic behavior of arsenate species and their weaker binding affinity to the functional groups of NHA compared to divalent cations like Cu^{2+} or Zn^{2}. Figure 6 illustrates the comparative metal concentrations across different treatments (HA1–HA5 and NANO1–NANO4), where a clear dose-dependent trend was observed for Cu and Zn. In contrast, Mg and As showed nonlinear behaviors, possibly due to competition for adsorption sites or saturation effects. These findings demonstrate that NHA can achieve rapid and significant immobilization of heavy metals within 30 days.


Cu mg/kg (30 Days)
Copper Concentration (mg/kg)
7
6
5
4
3
2
1
CK
HA1
HA2
HA3
HA4
HA5
NANO1
NANO2
NANO3
NANO4
Treatment Groups


Zn mg/kg (30 Days)
Zinc Concentration (mg/kg)
16
14
12
10
8
6
CK
HA1
HA2
HA3
HA4
HA5
NANO1
NANO2
NANO3
NANO4
Treatment Groups


As mg/kg (30 days)
Arsenic Concentration (mg/kg)
0.6
0.5
0.4
0.3
CK
HA1
HA2
HA3
HA4
HA5
NANO1
NANO2
NANO3
NANO4
Treatment Groups

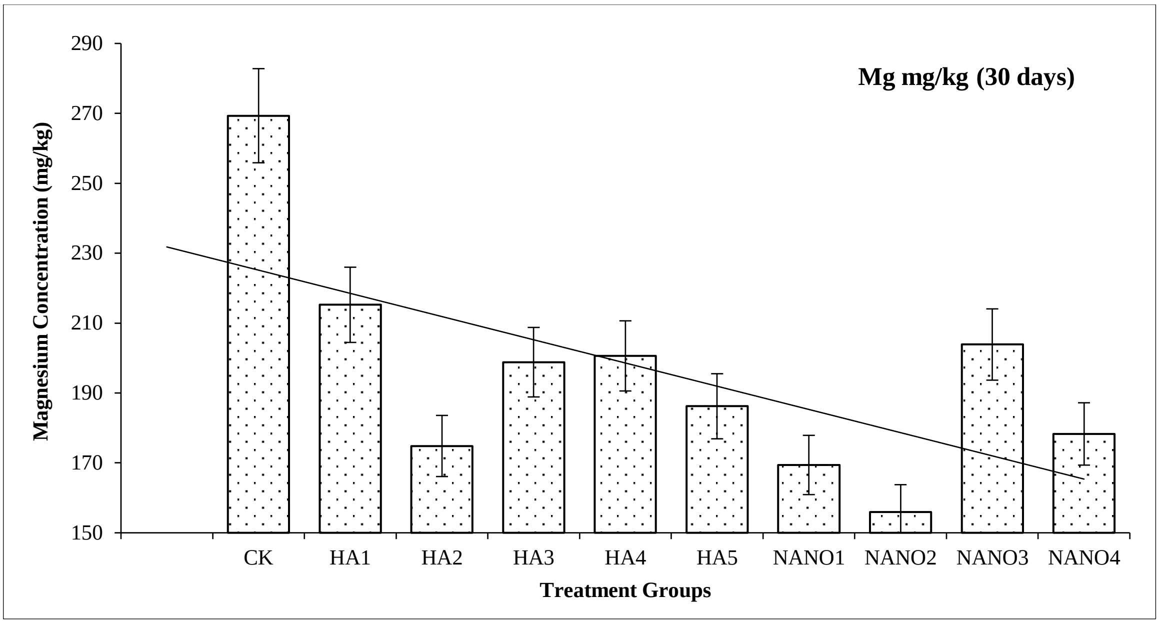


Figure 6. **Changes in heavy metal concentrations (Cu, Zn, As, Mg) after 30-day treatment with various HA and**

These findings confirm that NHA significantly improves the immobilization of heavy metals in polluted soil within just 30 days of application. Its enhanced surface reactivity, increased functional group density, and smaller particle size allow for more effective complexation and retention of contaminants. Among all treatments, NANO3 and NANO4 showed the most substantial reductions in Cu and Zn, respectively, while NANO2 was most effective for Mg removal. Although As exhibited the lowest removal efficiency, its reduction under NANO3 was still statistically significant. These results are summarized in Table 9, which presents the mean ± SD values for each metal, the percentage reduction achieved, and the statistical significance from ANOVA and Tukey HSD post hoc tests.

Table 9. **Heavy Metal Concentrations Before and After 30-Day Nano Humic Acid Treatment (Mean ± SD, n = 3)**

Metal	Initial Conc. (mg/kg)	Final Conc. (mg/kg, best NHA)	SD (mg/kg)	% Reduction	Best Treatment	p-value (ANOVA)	Significance
Cu	6.53 ± 0.38	1.562 ± 0.12	0.12	76.1%	NANO3	0.0008	***
Zn	15.006 ± 0.53	6.379 ± 0.35	0.35	57.5%	NANO4	0.0032	**
As	0.425 ± 0.026	0.370 ± 0.02	0.02	12.9%	NANO3	0.042	*
Mg	269.33 ± 9.42	155.97 ± 8.45	8.45	42.1%	NANO2	0.015	*

Note: Values are reported as mean ± standard deviation (SD); significance levels: $p < 0.05$ (*), < 0.01 (**), < 0.001 (***).

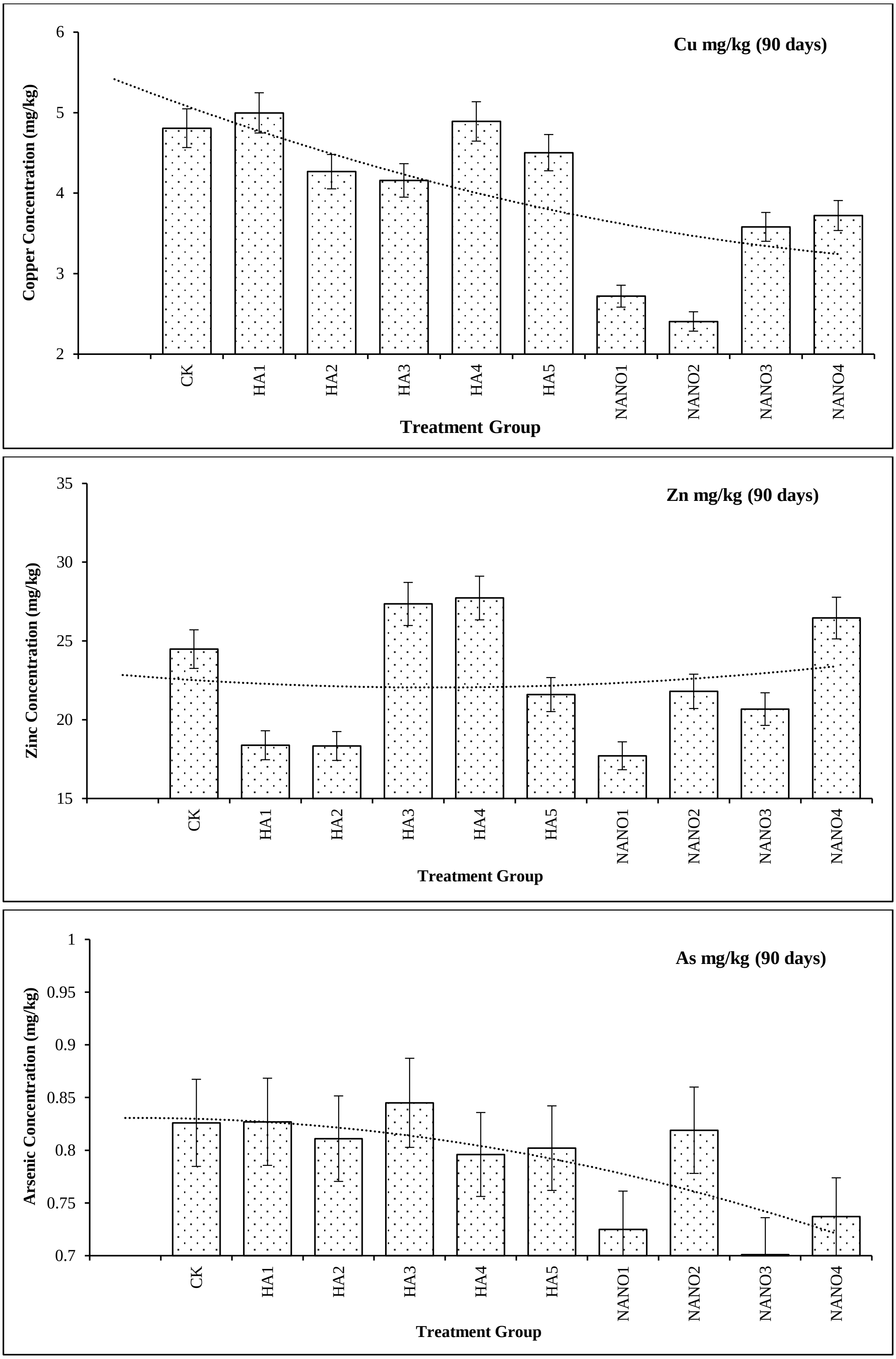
3-4- Changes of Heavy Metal Content in Contaminated Soil After 90 Days of Adsorption

After 90 days of treatment, nano humic acid (NHA) maintained significant adsorption capacity, indicating the material's sustained performance in contaminated soil environments. The concentration of magnesium (Mg) in the control group (CK) was 293.33 ± 9.65 mg/kg. Under the NANO1 treatment (12.5 g/kg), the Mg concentration decreased to 214.67 ± 10.12 mg/kg ($p = 0.021$), representing a 26.8% reduction. While higher dosages (NANO2–NANO4) also showed reduced Mg levels (242.33–229.33 mg/kg), the overall trend suggested a plateau, indicating possible saturation of available binding sites at higher application rates.

For copper (Cu), the most notable reduction was observed under NANO2 (25 g/kg), where concentrations dropped from 4.807 ± 0.39 mg/kg to 2.406 ± 0.15 mg/kg ($p = 0.0011$, ***). This result confirms the high affinity and stable complexation between Cu^{2+} ions and the functional groups on the NHA surface, consistent with FTIR-identified chelators such as –COOH and $–SO_3$ H. Notably, this strong interaction persisted over the extended 90-day period, demonstrating the long-term retention capability of NHA for divalent metal cations.

Arsenic (As) concentrations were moderately reduced from 0.826 ± 0.035 mg/kg in the CK to 0.701 ± 0.03 mg/kg under NANO2 ($p = 0.038$, *). Although the overall reduction (15.1%) was smaller than for Cu or Mg, it remains statistically significant. The limited reduction efficiency may stem from the anionic nature of arsenic species in soil, which can undergo electrostatic repulsion or face competition with other anions such as nitrate or sulfate. Zinc (Zn), which responded strongly in the 30-day trial, showed a more modest reduction over 90 days. Under NANO1, the concentration decreased from 24.483 ± 0.61 mg/kg to 17.712 ± 0.44 mg/kg (27.7% reduction, $p = 0.027$, *). Interestingly, increasing the NHA dose beyond 12.5 g/kg did not significantly enhance Zn removal, suggesting nonlinear adsorption behavior possibly influenced by Zn speciation or slower kinetic uptake over time.

The data presented in Figure 7 illustrate these overall trends, with Cu and Mg showing consistent dose-dependent reductions and Zn and As exhibiting diminishing returns at higher application rates. These findings underscore the importance of optimizing dosage levels, especially for field-scale applications where material cost must be balanced with removal efficiency. A comprehensive comparison of pre- and post-treatment concentrations, along with associated statistical metrics, is provided in Table 10.



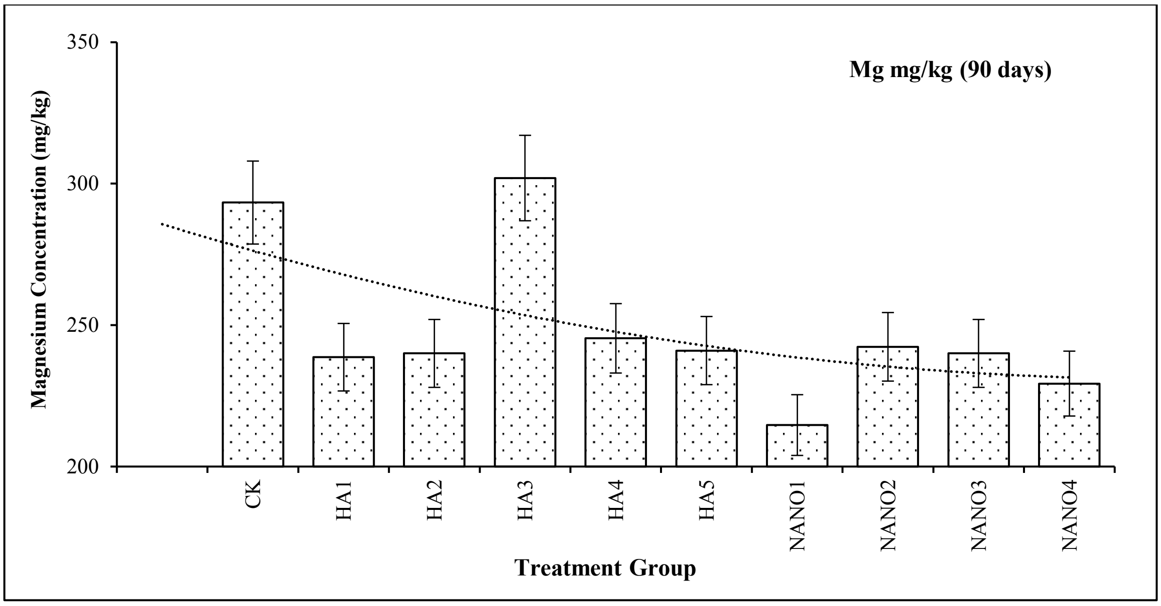


Figure 7. Heavy metal concentrations (Cu, Zn, As, Mg) in contaminated soil after 90-day treatment with various nano humic acid dosages

Table 10. Heavy Metal Concentrations Before and After 90-Day Nano Humic Acid Treatment (Mean ± SD, n = 3)

Metal	Initial Conc. (mg/kg)	Final Conc. (mg/kg, best NHA)	SD (mg/kg)	% Reduction	Best Treatment	p-value (ANOVA)	Significance
Cu	4.807 ± 0.39	2.406 ± 0.15	0.15	49.9%	NANO2	0.0011	***
Zn	24.483 ± 0.61	17.712 ± 0.44	0.44	27.7%	NANO1	0.0270	*
As	0.826 ± 0.035	0.701 ± 0.03	0.03	15.1%	NANO2	0.0380	*
Mg	293.33 ± 9.65	214.67 ± 10.12	10.12	26.8%	NANO1	0.0210	*

Note: Values are reported as mean ± standard deviation (SD); significance levels: $p < 0.05$ (*), < 0.01 (**), < 0.001 (***).

3-5- Changes of Ammonium Nitrogen and Nitrate Nitrogen Content in Contaminated Soil After 90 Days of Adsorption

In the control (CK) soil, the concentrations of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were recorded as 3.671 ± 0.15 mg/g and 2.332 ± 0.11 mg/g, respectively—typical of livestock-manure-contaminated soils. These elevated levels contribute to nitrate leaching, eutrophication, and overall soil quality degradation. Application of conventional humic acid at 12.5 g/kg (HA1) slightly decreased ammonium and nitrate nitrogen levels to 3.205 ± 0.14 mg/g and 1.875 ± 0.10 mg/g, respectively. However, nano humic acid (NANO1) at the same dosage led to significantly greater reductions: 2.545 ± 0.12 mg/g for NH_4^+-N and 1.369 ± 0.09 mg/g for NO_3^--N, indicating the enhanced sorption performance of the nanoform.

The best nitrate removal was achieved under the NANO2 treatment (25 g/kg), where NO_3^--N was reduced to 1.66 ± 0.08 mg/g, corresponding to a 55% decrease compared to CK ($p = 0.004$, ANOVA). This strong nitrate adsorption is likely facilitated by electrostatic interactions between NO_3^- anions and protonated amine groups on the NHA surface, along with entrapment within micro- or mesopores. Ammonium nitrogen under NANO2 also dropped to 1.86 ± 0.10 mg/g, reflecting a 20% reduction ($p = 0.048$). These results confirm that NHA exhibits a dual-function adsorption mechanism, offering significantly better nutrient immobilization than conventional humic acid, as shown in Figure 8.

Interestingly, while nitrate adsorption followed a relatively clear dose-response trend, ammonium adsorption plateaued beyond 25 g/kg. NANO3 and NANO4 yielded comparable or slightly lower reductions, suggesting possible ion-exchange saturation or weaker electrostatic interactions between NH_4^+ and NHA functional groups. These trends are supported by the correlation analysis in Table 11. A strong negative correlation was found between NHA material type and Cu ($r = –0.81$, $p < 0.001$), Mg ($r = –0.68$, $p < 0.05$), and As ($r = –0.70$, $p < 0.05$). Importantly, nitrate nitrogen (NO_3^--N) adsorption showed a strong positive correlation with Cu removal ($r = 0.87$, $p < 0.001$), suggesting a potential synergistic co-binding mechanism. In contrast, the correlation for ammonium nitrogen (NH_4^+-N) was weaker and statistically non-significant, consistent with its lower removal efficiency.

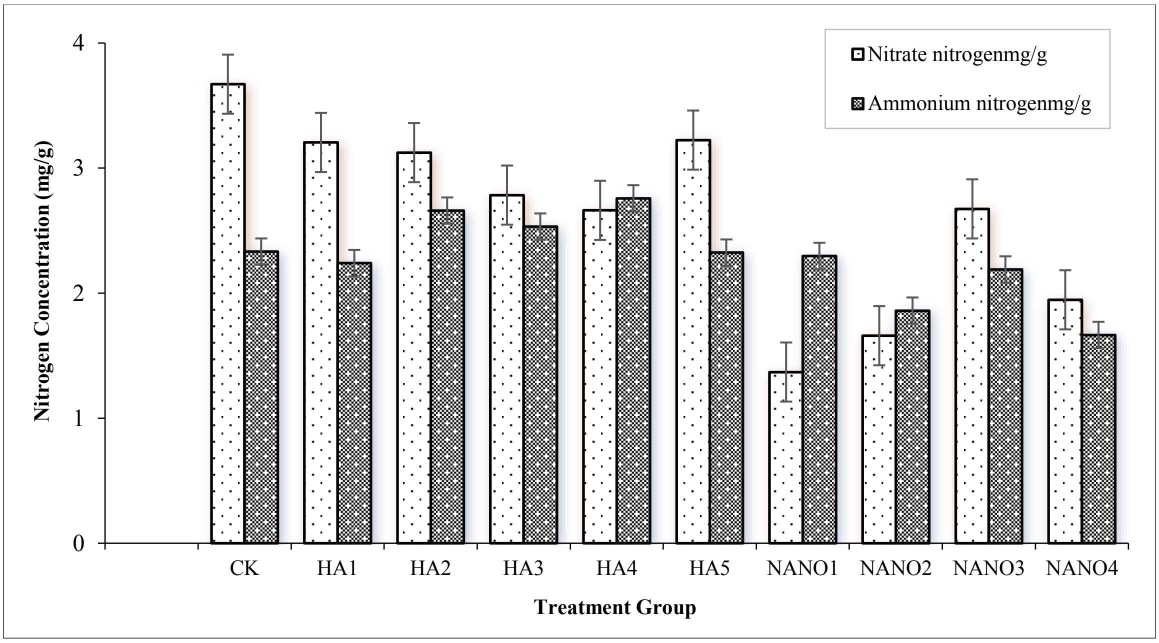


Figure 8. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) levels in contaminated soil after 90 days of treatment with different HA and NHA dosages

Interestingly, while nitrate adsorption followed a relatively clear dose-response trend, ammonium adsorption plateaued beyond 25 g/kg. NANO3 and NANO4 yielded comparable or slightly lower reductions, suggesting possible ion-exchange saturation or weaker electrostatic interactions between NH_4^+ and NHA functional groups. These trends are supported by the correlation analysis in Table 11. A strong negative correlation was found between NHA material type and Cu ($r = –0.81$, $p < 0.001$), Mg ($r = –0.68$, $p < 0.05$), and As ($r = –0.70$, $p < 0.05$). Importantly, nitrate nitrogen (NO_3^--N) adsorption showed a strong positive correlation with Cu removal ($r = 0.87$, $p < 0.001$), suggesting a potential synergistic co-binding mechanism. In contrast, the correlation for ammonium nitrogen (NH_4^+-N) was weaker and statistically non-significant, consistent with its lower removal efficiency.

Table 11. Correlation Coefficient Between Adsorbent Type, Application Rate, and Soil Pollutants (Heavy Metals and Nitrogen Species)

Variable	As	Cu	Zn	Mg
Application Rate	–0.37ns	–0.0061ns	0.36ns	–0.30ns
Material Type (HA vs. NHA)	–0.70*	–0.81***	–0.22ns	–0.68*
NO_3^- -N	0.51ns	0.87***	0.076ns	0.55ns
NH_4^+ -N	0.36ns	0.54ns	0.027ns	0.31ns

Note: *, **, and *** (significant, p<0.05, <0.01, and <0.001, respectively); ns (not significant, p≥0.05).

The nitrate nitrogen adsorption also showed a strong positive correlation with Cu removal ($r = 0.87$, $p < 0.001$), suggesting a potentially synergistic removal mechanism, possibly due to co-binding or co-localization on active NHA sites. In contrast, ammonium nitrogen correlations were modest and not statistically significant, aligning with its lower removal efficiency. Taken together, these findings confirm that NHA is more effective than traditional HA in simultaneously reducing nitrate and ammonium nitrogen in contaminated soils. The 25 g/kg application rate (NANO2) emerges as the most efficient dosage, offering the best balance between removal performance and material usage. The results also highlight the multifunctionality of NHA as a dual-action adsorbent for both metallic and nitrogenous pollutants in livestock-impacted agricultural soils.

4- Discussion

4-1- Comparison with Previous Studies

To contextualize the effectiveness of nano humic acid (NHA) observed in this study, a comparative analysis was conducted with key prior investigations involving humic-based adsorbents for heavy metal and nutrient removal in soil or aqueous systems. Table 12 presents a summary of relevant studies, including synthesis methods, target pollutants, experimental settings, and reported removal efficiencies.

Table 12. Comparison of Nano Humic Acid and Humic Acid Performance with Previous Studies

Study	Material Type	Target Pollutants	Medium	Max Removal Efficiency	Notable Findings
Cheng et al. [17]	Nanoscale HA (high-shear)	Cd, Cu, Zn	Wastewater	Cu: 71%, Zn: 60%	Fast kinetics; nanoscale improved metal binding
Liang [20]	NHA (acid-isolation)	Cd	Water	Cd: 80%	Good desorption; reusability potential
Kulikowska et al. [13]	Compost-derived HA	Cd, Cu, Zn, Ni	Soil	Cu: 45%, Zn: 41%	HA effective in aged soils
Çavusoglu et al. [14]	HA + phosphorus	Not specified	Soil (Gladiolus)	—	Improvement in soil fertility and metal tolerance
Our Study	NHA (high-shear + surfactant + acid precipitation)	Cu, Zn, Mg, As, NO_3^--N, NH_4^+-N	Contaminated agricultural soil	Cu: 76.1%, Zn: 57.5%, NO_3^--N: 55%	Dual-function performance in real soil, effective at 25–50 g/kg

Compared to the aqueous-based studies of Cheng et al. [17] and Liang [20], the current work expands the application of NHA to complex real-soil systems over a prolonged 90-day period. The Cu removal efficiency observed in this study (up to 76.1%) slightly exceeds the 71% reported in wastewater systems, likely due to the enriched sulfonic and amino functional groups enhancing binding affinity. Similarly, the nitrate nitrogen removal (55%) represents a novel dual-function capability not previously quantified in prior studies focused solely on metals. Kulikowska et al. [13] demonstrated moderate removal in compost-enriched soils using traditional HA, with lower performance compared to our nanoform due to surface area limitations. While Çavusoglu et al. [14] confirmed general benefits of HA in improving soil quality, their study did not quantify pollutant removal, emphasizing the novelty and practical relevance of the current findings in simultaneously addressing metal and nitrogen pollution.

4-2- Optimal Dosage Justification

While higher NHA dosages (e.g., 50 g/kg or 100 g/kg) occasionally yielded slightly greater removal of certain heavy metals at the 30-day mark, such as Zn under NANO4 and Cu under NANO3, these improvements were often marginal and not sustained over the 90-day period. For instance, Cu removal at 90 days was highest under NANO2 (25 g/kg), and nitrate and ammonium reductions also peaked at this dosage. In several cases, increasing the dosage beyond 25 g/kg led to plateau effects, likely due to saturation of active adsorption sites or diminishing diffusion efficiency. Moreover, higher dosages did not provide significantly better removal of Mg or arsenic, and in some cases, performance declined slightly. Considering both adsorption efficiency and material usage, the 25 g/kg dosage (NANO2) was therefore determined to be the optimal balance between performance and cost-effectiveness. This finding is particularly important for potential field-scale applications, where minimizing input cost is crucial.

4-3- Study Limitations and Future Work

While the current study demonstrated that nano humic acid (NHA) effectively adsorbed heavy metals and nitrogen compounds under field-simulated conditions, it did not evaluate the potential for desorption or remobilization of bound metals under changing environmental factors such as pH fluctuations or increased ionic strength. These conditions can significantly impact the long-term stability of NHA–metal complexes, particularly in acidic or saline-alkaline soils. Future studies should examine desorption behavior and leaching risks under variable conditions to ensure the environmental safety and durability of NHA-based remediation strategies.

5- Conclusion

This study demonstrates that nano humic acid (NHA), synthesized through high-shear and acid-precipitation techniques, offers significant advantages over conventional humic acid (HA) in remediating heavy metal and nitrogen pollution in livestock-manure-contaminated soils. Structural characterization through FTIR revealed the introduction of new functional groups, including amino and ester moieties, which enhanced the binding affinity of NHA for various contaminants. SEM and particle size analysis confirmed that nano-scaling improved dispersibility and surface area, with particles predominantly in the 50–90 nm range. The adsorption experiments showed that within 30 days, NHA significantly reduced concentrations of Cu, Zn, Mg, and As, outperforming HA across all application rates. After 90 days, the adsorption performance remained strong, particularly for Cu and Mg, while a plateau effect was observed for Zn and As at higher dosages. In addition, NHA exhibited notable efficiency in adsorbing nitrate nitrogen (up to 55% removal under $NANO_2$ treatment), whereas HA showed negligible effects. Although ammonium nitrogen adsorption was less pronounced, NHA still outperformed HA, especially at 25 g/kg. Overall, the optimal dosage of NHA was determined to be 25 g/kg, balancing effectiveness and material efficiency. These findings provide a robust theoretical and practical foundation for applying NHA as a dual-function, eco-friendly soil amendment in the remediation of metal- and nitrogen-contaminated agricultural lands, particularly in vulnerable regions such as the Yellow River basin and surrounding hilly areas. The study contributes to sustainable pollution control strategies and supports the ecological rehabilitation of degraded farmlands.

6- Declarations

6-1- Author Contributions

.Conceptualization, Q.S. and M.A.B.K.; methodology, Q.S.; validation, M.S.Y., Q.S., and K.H.; formal analysis, K.H.; investigation, K.H.; resources, Y.C. and Y.C.; data curation, Y.C. and Y.C.; writing—original draft preparation, Q.S.; writing—review and editing, M.A.B.K.; visualization, M.S.Y.; supervision, M.A.B.K.; project administration, M.A.B.K. All authors have read and agreed to the published version of the manuscript.

6-2- Data Availability Statement

The data presented in this study are available on request from the corresponding author.

6-3- Funding

This work was supported by Jiangsu Agriculture Science and Technology Innovation Fund (Grant Numbers: CX(23)2003).

6-4- Institutional Review Board Statement

Not applicable.

6-5- Informed Consent Statement

Not applicable.

6-6- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

7- References

[1] Tang, L. (2016). Effects of composting conditions on carbon losses and humus formation during the composting process. Master Thesis, Hangzhou Normal University, Hangzhou, China.

[2] Xu, W. P., Chen, T. B., Liu, J. L., & He, Y. Q. (2004). Environmental pollution, comprehensive prevention and control tactics of the scale and intensify poultry farming. Environmental Science, 25(S1), 105-108.

[3] Su, Y. (2006). Research of countermeasures on waste treating of intensive livestock and poultry farms in China. Chinese Journal of Eco-Agriculture, 14(2), 15-18.

[4] Liao, Q., Wei, G., Jiang, Z., Xing, Y., Huang, D., & Li, Y. (2014). Research progress on resource utilization of livestock and poultry manure. Agricultural Science & Technology, 15(1), 105.

[5] Zhou, Y., Li, S., Yang, F., Guan, Q., & Zhang, N. (2025). Distribution and risk assessment of heavy metals in the upper, middle, and lower reaches of the Yellow River wetlands. Limnology, 26(2), 349-363. doi:10.1007/s10201-025-00780-1.

[6] Cai, T., You, L., Yang, X., Hao, S., Shao, Q., Wang, H., ... & Chen, Y. (2023). Fertilization of peach for yield and quality, and optimization of nitrogen application rates in China: A meta-analysis. Scientia Horticulturae, 313, 111917. doi:10.1016/j.scienta.2023.111917.

[7] Liu, Q., Hu, J., Gong, S., Luo, F., & Yang, G. (2024). Enrichment characteristics and ecological risk assessment of heavy metal Cd in soil and sediment along the Yangtze River in Nanjing, Jiangsu Province. Geological Bulletin of China, 43(5), 756–765. doi:10.12097/gbc.2022.12.008.

[8] He, Z. (2020). Sustainable development of livestock and poultry scale-breeding based on integration control of resource losses and external environmental costs. Environmental Progress & Sustainable Energy, 39(6), e13528. doi:10.1002/ep.13528.

[9] Awual, M. R., Hasan, M. M., Asiri, A. M., & Rahman, M. M. (2019). Cleaning the arsenic (V) contaminated water for safe-guarding the public health using novel composite material. Composites Part B: Engineering, 171, 294-301. doi:10.1016/j.compositesb.2019.05.078.

[10] Wang, K. R. (1997). China's farmland cadmium pollution situation and governance utilization. Journal of Agro-Environmental Science, 16(6), 274-278..

[11] Ding-qing, Y. A. N. G., & Shao-qing, F. U. (2000). Effect of application of pig dung with high Zn on soil contamination. Sichuan Environment, 2, 30-34.

[12] Ding, G. A. O., Tong-bin, C. H. E. N., Bin, L. I. U., Yuan-ming, Z. H. E. N. G., Guodi, Z. H. E. N. G., & Yan-Xia, L. I. (2006). Releases of pollutants from poultry manure in China and recommended strategies for the pollution prevention. Geographical Research, 25(2), 311-319.

[13] Kulikowska, D., Gusiatin, Z. M., Bułkowska, K., & Klik, B. (2015). Feasibility of using humic substances from compost to remove heavy metals (Cd, Cu, Ni, Pb, Zn) from contaminated soil aged for different periods of time. Journal of Hazardous Materials, 300, 882–891. doi:10.1016/j.jhazmat.2015.08.022.

[14] Çavusoglu, M. N., Türkoğlu, N., & Özdemir, F. A. (2017). The effects of phosphorus and humic acid on some soil properties flowers quality of Gladiolus. Progress in Nutrition, 19, 150–155. doi:10.23751/pn.v19i1-S.5822.

[15] Martyniuk, H., & Więckowska, J. (2003). Adsorption of metal ions on humic acids extracted from brown coals. Fuel Processing Technology, 84(1–3), 23–36. doi:10.1016/S0378-3820(02)00246-1.

[16] Cheng, L., Xu, L., Liu, W., Zhang, B. L., & Liu, G. J. (2014). Efficient removal of phenol wastewater using nanoscale humic acid adsorbent in ultrasound aided. Huaxue Gongcheng/Chemical Engineering (China), 42(9), 6–11. doi:10.3969/j.issn.1005-9954.2014.09.002.

[17] Cheng, L., Zhang, B., Hou, C., Chen, K., Wang, J., & Shi, Y. (2012). Preparation and characterization of nanoscale humic acid under high shearing condition. Huagong Xuebao/CIESC Journal, 63(8), 2648–2654. doi:10.3969/j.issn.0438-1157.2012.08.044.

[18] Cheng, L., Hou, C., Xu, L., Luo, T., Zhang, B., & Liu, G. (2016). Dynamic adsorption and de-sorption characteristics of wastewater containing cadmium ion on nanoscale humic acid. Huagong Xuebao/CIESC Journal, 67(4), 1348–1356. doi:10.11949/j.issn.0438-1157.20151099.

[19] Erhayem, M., & Sohn, M. (2014). Effect of humic acid source on humic acid adsorption onto titanium dioxide nanoparticles. Science of the Total Environment, 470–471, 92–98. doi:10.1016/j.scitotenv.2013.09.063.

[20] Liang C., (2012). Study on preparation of nanoscale humic acid by alkali-solution and acid-isolation, Master Thesis, Zhengzhou University, Zhengzhou, China.

[21] Yuan, Y., Fang, M., Liu, Y., Zhang, B., Chen, T., Jin, J., Zhuang, L., Yang, T., & Zhang, J. (2025). Enhanced soil remediation with iron-synthetic humic-like acid composites: a trade-off between metal immobilization efficacy and micro-ecological health. Journal of Environmental Management, 389, 126045. doi:10.1016/j.jenvman.2025.126045.

[22] Liu, Y., Zhou, D., Huang, P., Yang, C., Zhang, J., Wang, H., Cheng, Q., Liu, Y., Gao, C., Ma, J., Lin, H., & Ma, J. (2025). Humic acid-supported nanoscale zero-valent iron for sustainable cadmium remediation and crop safety in farmland soil. Journal of Hazardous Materials, 492, 138109. doi:10.1016/j.jhazmat.2025.138109.

[23] Xue, S., Hu, Y., Wan, K., & Miao, Z. (2024). Exploring Humic Acid as an Efficient and Selective Adsorbent for Lead Removal in Multi-Metal Coexistence Systems: A Review. Separations, 11(3), 80. doi:10.3390/separations11030080.

[24] Wang, M., Song, G., Zheng, Z., Song, Z., Mi, X., Hua, J., & Wang, Z. (2024). Effect of humic substances on the fraction of heavy metal and microbial response. Scientific Reports, 14(1), 11206. doi:10.1038/s41598-024-61575-5.

[25] Masmoudi, S., Abid, W., Medhioub, K., & Ammar, E. (2024). Compost derived from olive mill cake: Effects on isohumic soil quality based on humic acids characterization. Heliyon, 10(16). doi:10.1016/j.heliyon.2024.e36456.

[26] Samani, M., Ahlawat, Y. K., Golchin, A., Alikhani, H. A., Fathi-Gerdelidani, A., Ahlawat, U., Malik, A., Panwar, R., Maan, D. S., Ahmed, M., Thakur, P., & Mishra, S. (2024). Nano silica-mediated stabilization of heavy metals in contaminated soils. Scientific Reports, 14(1), 20496. doi:10.1038/s41598-024-69182-0.

[27] Anitha, N., Sheeba, S., Prabhaharan, J., Vallal Kannan, S., Amutha, R., & Sivasankari, B. (2025). Humic Acid: A Natural Enhancer of Soil Quality and Crop Productivity. Communications in Soil Science and Plant Analysis, 56(19), 2831–2843. doi:10.1080/00103624.2025.2527384.

[28] Zhang, Z., Chen, Q., Xu, K., Zhang, K., Zhang, M., Qi, Y., Zhang, W., Liu, Y., Wei, Z., & Liu, Z. (2024). Selective Modifier-Assisted Humic Acid Extraction: Implications for Soil Quality Enhancement. Environmental Science and Technology, 58(22), 9896–9907. doi:10.1021/acs.est.3c10713.